Table 2:
Allergenic potency ($P_{rel.}$) of the recombinant Phl p 5b mutants as compared with that of recombinant and native Phl p 5b using the allergic patient serum pool Bor 18/100

Inhibitor	Inhibition value[1] [mol/l]		Allergenic potency (P_{rel})[2]	
	25%	50%	25%	50%
n Phl p 5b	3.3×10^{-10}	4.2×10^{-9}	1.000	1.000
r Phl p 5b	2.0×10^{-10}	5.0×10^{-9}	1.709	0.8410
PM1	4.5×10^{-10}	1.2×10^{-8}	0.739	0.3490
PM3	2.0×10^{-10}	4.8×10^{-9}	1.641	0.8640
DM1	8.6×10^{-9}	2.8×10^{-8}	0.039	0.0015
DM2	8.3×10^{13}	2.3×10^{38}	4.0×10^{-23}	1.8×10^{-45}
DM3	1.2×10^{-8}	4.1×10^{-5}	0.028	0.0001
DM2*	5.0×10^{23}	2.3×10^{66}	5.7×10^{-34}	2.0×10^{-75}

[1] Inhibition values: Concentrations of the inhibitors at 25% and 50% inhibition, respectively

[2] Allergenic potency: Relative to native Phlp5b at 25% and ~~50% inhibition, respectively~~ 50% inhibition, respectively

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Table 3:
Allergenic potency ($P_{rel.}$) of the recombinant Phl p 5b mutants as compared with that of recombinant and native Phl p 5b using the allergic patient serum pool We 6/97

Inhibitor	Inhibition value[1] [mol/l]		Allergenic potency (P_{rel})[2]	
	25%	50%	25%	50%
n Phl p 5b	5.1×10^{-10}	6.1×10^{-9}	1.000	1.000
r Phl p 5b	3.0×10^{-10}	1.4×10^{-8}	1.697	0.4400
PM1	1.2×10^{-9}	$1.2 \cdot 10^{-7}$	0.415	0.0510
PM3	8.3×10^{-10}	$3.0 \cdot 10^{-3}$	0.611	0.2030
DM1	2.3×10^{-8}	1.7×10^{-5}	0.022	0.0004
DM2	1.9×10^{9}	2.7×10^{21}	2.6×10^{-15}	2.3×10^{-30}
DM3	$5.1 \cdot 10^{-9}$	$2.9 \cdot 10^{-8}$	0.099	0.0020
DM2*	4.6×10^{-7}	$1.5 \cdot 10^{-3}$	0.001	4.0×10^{-8}

[1] Inhibition values: Concentrations of the inhibitors at 25% and 50% inhibition, respectively

[2] Allergenic potency: Relative to native Phlp5b at 25% and ~~50% inhibition, respectively~~ 50 inhibition, respectively

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Table 4:
Allergenic potency ($P_{rel.}$) of the recombinant Phl p 5b mutants as compared with that of recombinant and native Phl p 5b using the individual allergic patient serum II/3

Inhibitor	Inhibition value[1] [mol/l]		Allergenic potency (P_{rel})[2]	
	25%	50%	25%	50%
n Phl p 5b	5.1×10^{-10}	$5.9 \cdot 10^{-9}$	1.000	1.000
r Phl p 5b	$5.6 \cdot 10^{-10}$	$1.4 \cdot 10^{-8}$	0.9030	0.4190
PM1	8.6×10^{-10}	1.9×10^{-8}	0.5950	0.3140
PM3	5.5×10^{-10}	1.5×10^{-8}	0.9220	0.3990
DM1	1.2×10^{-8}	$1.7 \cdot 10^{-8}$	0.0420	0.0035
DM2	$6.6 \cdot 10^{-10}$	$5.2 \cdot 10^{27}$	7.7×10^{-20}	1.1×10^{-38}
DM3	1.1×10^{-6}	0.032	0.0004	1.8×10^{-7}
DM2*	2.1×10^{-6}	0.010	0.0002	5.9×10^{-7}

[1]: Inhibition values: Concentrations of the inhibitors at 25% and 50% inhibition, respectively

[2]: Allergenic potency: Relative to native Phlp5b at 25% and ~~Allergenic potency: Relative to native Phlp5b at 25% and~~ 50% inhibition, respectively

Table 5:
Allergenic potency ($P_{rel.}$) of the recombinant Phl p 5b mutants as compared with that of recombinant and native Phl p 5b using the individual allergic patient serum II/12

Inhibitor	Inhibition value[1] [mol/l]		Allergenic potency (P_{rel})[2]	
	25%	50%	25%	50%
n Phl p 5b	$5.2 \cdot 10^{-10}$	$6.8 \cdot 10^{-9}$	1.000	1.000
r Phl p 5b	$8.7 \cdot 10^{-10}$	$7.3 \cdot 10^{-8}$	0.597	0.093
PM1	$1.3 \cdot 10^{-9}$	$8.3 \cdot 10^{-8}$	0.391	0.082
PM3	$1.3 \cdot 10^{-9}$	$9.1 \cdot 10^{-8}$	0.389	0.075
DM1	$1.5 \cdot 10^{-5}$	58.0	$3.4 \cdot 10^{-5}$	$1.0 \cdot 10^{-10}$
DM2	$3.8 \cdot 10^{10}$	$4.4 \cdot 10^{30}$	$1.4 \cdot 10^{-19}$	$1.6 \cdot 10^{-39}$
DM3	$4.5 \cdot 10^{-8}$	0.0001	0.012	$5.7 \cdot 10^{-5}$
DM2*	196.0	$7.4 \cdot 10^{14}$	$2.6 \cdot 10^{-12}$	$9.2 \cdot 10^{-35}$

[1] Inhibition values: Concentrations of the inhibitors at 25% and 50% inhibition, respectively

[2] ~~Allergenic potency: Relative to native Phl p5b at 25% and~~ Allergenic potency: Relative to native Phl p5b at 25% and 50% inhibition, respectively

Table 6:
Allergenic potency ($P_{rel.}$) of the recombinant Phl p 5b mutants as compared with that of recombinant and native Phl p 5b using the individual allergic patient serum II/17

Inhibitor	Inhibition value[1] [mol/l]		Allergenic potency (P_{rel})[2]	
	25%	50%	25%	50%
n Phl p 5b	2.2×10^{-10}	2.6×10^{-9}	1.000	1.000
r Phl p 5b	~~2.2×10^{-10}~~ $21 < 10^{-10}$	$4.7 \cdot 10^{-9}$	1.045	0.5450
PM1	6.4×10^{-10}	2.2×10^{-9}	0.336	0.1190
PM3	2.5×10^{-10}	5.5×10^{-9}	0.855	0.4680
DM1	$6.5 \cdot 10^{-9}$	2.0×10^{-8}	0.033	0.0010
DM2	73.9	$6.4 \cdot 10^{19}$	$2.9 < 10^{-12}$	$4.1 < 10^{-29}$
DM3	$5.6 \cdot 10^{-9}$	$5.0 < 10^{-8}$	0.038	0.0005
DM2*	0.0004	11675.0	$5.3 < 10^{-7}$	$2.2 < 10^{-13}$

[1] Inhibition values: Concentrations of the inhibitors at 25% and 50% inhibition, respectively

[2] Allergenic potency: ~~Relative to native Phl p 5b at 25% and 50% inhibition, respectively~~ Allergenic potency: Relative to native Phl p5b at 25% and

Example 5

Reduced histamine release from basophils due to the rPhl p 5b mutants

The ability of the point mutant PM3 which was prepared, and of deletion mutants DM1, DM2, DM2* and DM3, to release histamine from basophils was tested and compared with that of the wild type rPhl p 5b.

Before the histamine release test was carried out, the basophilic leucocytes from the EDTA blood of an allergic patient (PS-W) were first of all enriched by means of dextran sedimentation and then adjusted to a final concentration of 100,000 basophils/ml. In order to release histamine from the basophils, 200 µl of the cell suspension were in each case incubated, at 37°C for 40 min, with 50 µl of antigen solution. For this, the rPhl p 5b and the mutants were employed in varying concentrations (of 10^{-5} - 10^{-12} M). The histamine which was released was determined in the respective supernatants using the Pharmacia methylhistamine RIA in accordance with the manufacturer's instructions.

In the histamine release test, all the recombinant proteins investigated described the typical bell-shaped curve as their concentrations increased (Fig. 6). The point mutant did not show any significant differences as compared with the wild type rPhl p 5b in its ability to release histamine. The concentrations of the deletion mutants DM3, DM1 and DM2 which were required to bring about a 30% histamine release were 3-fold, 20-fold and 500-fold higher, respectively. The deletion mutants therefore unambigously exhibit a decreased ability to release histamine from basophils.

Recapitulative assessment of the results described in Examples 1 - 7

The mapping of the epitopes of the main allergen Phl p 5b which are recognized by T helper cells from patients who are allergic to grass pollen has demonstrated that the T cell epitopes of the individual T cell clones (TCLs) are distributed over the entire sequence of the Phl p 5b. However, **3 immunodominant T cell-reactive regions** which are recognized by 85% of the TCCs can be defined without difficulty (Example 1). It was possible to produce recombinant Phl p 5b mutants by means of point mutations (Example 2) and by means of deletion mutations (Example 3). The IgE reactivity of the point mutants (PM1 and PM3), as measured in the EAST inhibition test (Example 4), does not differ significantly from that of the wild-type Phl p 5b. While the IgE reactivity of the deletion mutants DM1 and DM3 is greatly reduced, it is still detectable. By contrast, the IgE binding of mutants DM2 and DM2' is very greatly reduced. This gradual decrease in the allergenicity of the rPhl p 5b mutants is also confirmed by the histamine release test using spec. IgE-loaded basophils from the blood of allergic patients (Example 5). The testing of the rPhl p 5b mutants with epitope-mapped T cell clones confirms that the point mutations and deletion mutations react with, or fail to stimulate, the TCCs in the expected manner (Example 6). Using oligoclonal T cell lines which were established from the blood of patients who are allergic to grass pollen by means of stimulation with Phl p 5, it was possible to demonstrate that the mutants are able to stimulate oligoclonal TCLs of this nature (Example 7). Taking the results of the reduction in allergenicity and the retention of the T cell stimulation together, the mutants, particularly the deletion mutants, constitute recombinant allergen variants which are potentially suitable for specific immunotherapy.

The following examples relate to pharmaceutical preparations:

Example A: Injection vials

A solution of 100 g of an active compound or of an active compound mixture based on the modified recombinant allergens and 5 g of disodium hydrogen phosphate in 3 l of doubly distilled water is adjusted to pH 6.5 with 2N hydrochloric acid, sterilized by filtration, aliquoted into injection vials and lyophilized under sterile conditions; the vials are then sealed in a sterile manner. Each injection vial comprises 5 mg of active compound.

Example B: Suppositories

A mixture of 20 g of an active compound in the form of the modified recombinant allergens together with 100 g of soya bean lecithin and 1400 g of cocoa butter is melted, poured into moulds and allowed to cool. Each suppository comprises 20 mg of active compound.

Example C: Solution

A solution of 1 g of an active compound in the form of the modified recombinant allergens, 9.38 g of $NaH_2PO_4 \cdot 2\,H_2O$, 28.48 g of $Na_2HPO_4 \cdot 12\,H_2O$ and 0.1 g of benzalkonium chloride is prepared in 940 ml of doubly distilled water. The solution is adjusted to pH 6.8, made up to 1 l and sterilized by irradiation. This solution can be used in the form of eye drops.

Example D: Ointment

500 mg of an active compound in the form of the modified recombinant allergens are mixed with 99.5 g of yellow soft paraffin under aseptic conditions.

Example E: Tablets

A mixture of 1 kg of active compound in the form of the modified recombinant allergens, 4 kg of lactose, 1.2 kg of potato starch, 0.2 kg of talc and 0.1 kg of magnesium stearate is compressed into tablets in the customary manner such that each tablet comprises 10 mg of active compound.

Example F: Coated tablets

Tablets are compressed in analogy with Example E and are then coated, in a customary manner, with a coating consisting of sucrose, potato starch, talc, gum tragacanth and dye.

Example G: Capsules

2 kg of active compound in the form of the modified recombinant allergens are aliquoted, in a customary manner, into hard gelatin capsules such that each capsule comprises 20 mg of the active compound.

Example H: Ampoules

A solution of 1 kg of active compound in the form of the modified recombinant allergens in 60 l of doubly distilled water is sterilized by filtration, aliquoted into ampoules and lyophilized under sterile conditions; the ampoules are then sealed in a sterile manner. Each ampoule comprises 10 mg of active compound.

Example I: Inhalation spray

14 g of active compound in the form of the modified recombinant allergens are dissolved in 10 l of an isotonic solution of NaCl and the solution is aliquoted into commercially available spraying vessels which are fitted with a pump mechanism. The solution can be

sprayed into the mouth or the nose. One spraying stroke (approximately 0.1 ml) corresponds to a dose of approximately 0.14 mg.